fertility, insertion mutants, where transcription of the corresponding gene was abolished, were hypersensitive to ABA. Our results show, that the COS system is suitable for the identification of novel ABA regulatory factors.

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Expression and epigenetic studies of MDR1 genes in drug-resistant rat cells

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The overexpression of multidrug resistance 1 protein (MDR1, Abcb1 or P-glycoprotein), a member of the ABC (ATP Binding Cassette) transporter superfamily, can be responsible for the decreased efficiency of chemotherapeutic drugs in tumour cells. MDR1 is an energy-dependent transporter that is able to extrude cytotoxic agents from the cell. In the presence of these drugs MDR1 expression is up-regulated by different mechanisms, though the molecular background of increased MDR expression is mostly unknown. Recent studies suggested that epigenetic modifications (e.g. histone acetylation, methylation) might play an important role in this process.

The aim of our study was to reveal epigenetic modifications responsible for the increased MDR1 level in multidrug resistant cell lines.

We studied the MDR expression in drug resistant rat hepatoma cells kindly provided by A. Venetianer. The cell lines we used in our experiments were a drug sensitive parental rat hepatoma cell line (D12), a medium (col500) and a highly (col1000) drug-resistant variant of it, selected using increasing concentrations of colchicine (Pirity 1996).

In contrast to humans, rodents have two MDR1 isoforms: Abcb1a and Abcb1b. First, we determined the expression of these genes and found that the mRNA levels of both Abcb1a and Abcb1b were increased in the drug resistant cell lines compared to the parental D12. A potential reason for the elevated expression of the Abcb1 genes is gene amplification. Indeed, we observed an increase in the copies of the Abcb1 genes in the col1000 cell line, however our data suggested that gene amplification was not the (only) reason for the overexpression of Abcb1 genes in the resistant cells.

Next we studied the possible role of histone acetylation in the increased expression of Abcb1 genes. For this, we treated the cells with histone deacetylase inhibitors (Na-butirate and trichostatin A) to maintain the acetylated state of histones. As a consequence of the treatment, the acetylation of H3 and H4 histones increased. Surprisingly, Abcb1a and Abcb1b genes responded to the treatment in an opposite way: the expression of Abcb1a was decreased, while the expression of Abcb1b was increased in cells treated with histone deacetylase inhibitors. Since acetylation of histone 3 lysin 9 and 14 (H3K9ac and H3K14ac) have been shown to play key roles in the regulation of chromatin structure and function, and are linked to transcriptional activation, next we focused on these modifications in order to determine whether they play a role in the differential expression of Abc1a and b genes. Using chromatin immunoprecipitation we determined the H3K9ac and H3K14ac levels at the transcriptional start sites and at upstream regulatory regions of both genes. We found elevated H3K9 and H3K14 acetylation in the col500 resistant cell line in all tested Abcb1 regions. In contrast with that, the acetylation levels of these histones were comparable in the parental D12 and in the other resistant (col1000) cell lines. After histone deacetylase inhibitor treatment, H3K9 and H3K14 acetylation increased in all tested regions of both genes, contrary that, their expression changed in opposite directions.

Since HDAC inhibitors changed the expression levels of Abcb1 genes, we wondered whether this treatment affected the drug efflux capacity of the cells. To answer this question we compared the accumulation of a fluorescent cytotoxin, a substrate of MDR1, in treated and untreated cells. As expected, we detected an increased efflux activity in the drug resistant col500 and col1000 cells; however, TSA-treatment did not influence significantly this process.

In conclusion, our data suggest that elevated Abcb1 gene expression is not always coupled to histone acetylation changes and conversely, the H3K9 and H3K14 acetylation levels do not necessarily predict the expression level of the Abcb1 genes. Thus, further histone acetylation sites and other histone modifications need to be examined to understand the complex regulation of MDR by mechanisms affecting chromatin structure.

Pirity M, Hevér-Szabó A, Venetianer A (1996) Overexpression of P-glycoprotein in heat- and/or drug-resistant hepatoma variants. Cytotechnology 19(3):207-14.

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